

## Karyomorphology of Two Genera in Stemonaceae

KAZUO OGINUMA<sup>1</sup>, KAZUMI HORIUCHI<sup>1</sup>  
and TATSUNDO FUKUHARA<sup>2</sup>

<sup>1</sup>Faculty of Human Life and Environmental Science, Kochi Women's University, Kochi 780-8515, Japan;

<sup>2</sup>Department of Natural Environmental Science, Faculty of Human and Environmental Studies, Kyoto University, Kyoto 606-8501, Japan; <sup>2</sup>Present Address: Department of Environmental Education, Fukuoka University of Education, Munakata 811-4146, Japan

Chromosome numbers were reported in five species of Stemonaceae, i.e., *Croomia heterosepala* ( $2n=24$ ), *C. japonica* ( $2n=24$ ), *Stemona japonica* ( $2n=14$ ), *S. sessilifolia* ( $2n=14$ ) and *S. tuberosa* ( $2n=14$ ). Karyotypes were also described in these species exclusive of *S. tuberosa*. In the classification of the centromeric position, *C. heterosepala* and *C. japonica* are identical to each other, but *S. japonica* and *S. sessilifolia* are slightly different. The present and earlier reports confirm that the three genera of Stemonaceae (*s.str.*) have different base number:  $x=12$  in *Croomia*,  $x=7$  in *Stemona*, and  $x=9$  in *Stichoneuron*. With currently available phylogenetic hypotheses, the primitive base number of the family seems to be  $x=7$ .

Key words: *Croomia*, karyomorphology, *Stemona*, Stemonaceae

Stemonaceae is a small family with four genera, *Croomia* Torr. (3 spp., distributed in SE North America and E Asia), *Pentastemona* Steenis (2 spp., in Sumatra), *Stemona* Lour. (ca. 25 spp., in SE Asia through Malesia to N Australia), and *Stichoneuron* Hook. f. (2 spp., in SE Asia). The circumscription and affinities of Stemonaceae have long been controversial. *Pentastemona* has been often regarded as distant from the others (Stemonaceae *s.str.*) and suggested to be near *Trichopus* (Trichopodaceae), *Stenomeris* (Dioscoreaceae), *Tacca* (Taccaceae) or Zingiberales [reviewed in Duyfjes (1991, 1992) and Kubitzki (1998a)]. Stemonaceae *s.str.* has also been positioned variously in previous taxonomic systems [reviewed in Kubitzki (1998b)]. Recent DNA sequence-based phylogenetic analyses (Behnke *et al.* 2000, Caddick *et al.* 2000, Chase *et al.* 2000, Fuse & Tamura 2000, Qiu *et. al.* 1999, Soltis *et al.*

2000, for older studies, see the articles cited here) have placed Stemonaceae within Pandanales composed of Cyclanthaceae, Pandanaceae, Stemonaceae and Velloziaceae Blume (including Acanthochlamydaceae; Behnke *et al.* 2000) [plus perhaps *Sciaphila* (Triuridaceae); Chase *et al.* 2000] with high confidence parameters. Within Stemonaceae, *Pentastemona* comes sister to Stemonaceae *s.str.* (represented by *Stemona* and *Stichoneuron*) based on *rbcL* and *atpB* sequences (Caddick *et al.* 2000).

Although chromosome numbers have been reported in each of the genera of Stemonaceae (Table 2), two different numbers have been reported in *Croomia*, and only one species have been covered for the other three genera. Karyotypic description is virtually lacking.

In this paper, we report chromosome numbers and karyotypes of two species of

TABLE I. Studied taxa of *Croomia* and *Stemona*, and their collection data

Species	Collection data
<i>Croomia heterosepala</i> (Backer) Okuyama	Kochi Pref. Takaoka-gun, Sakawa-cho <i>Oginuma</i> 99004
<i>C. japonica</i> Miq.	Kochi Pref. Nankoku-shi, Oko <i>Oginuma</i> 99005
<i>Stemona japonica</i> Miq.	Hyogo Pref. Shumoto-shi, Yura <i>Fukuhara STE-01</i>
<i>S. sessilifolia</i> Miq.	Nagasaki Pref. Nagasaki-shi, Hongouchi <i>Fukuhara STE-02</i>
<i>S. tuberosa</i> Lour.	Cultivated* <i>Fukuhara STE-03</i>
	Cultivated* <i>Fukuhara STE-04</i>
	Cultivated* <i>Fukuhara STE-05</i>

\* Kyoto Herbal Garden of Takeda Chemical Industries, LTD.

*Croomia* native to Japan and three cultivated species of *Stemona* (karyotype of *S. tuberosa* could not be determined), and summarize chromosomal information of the family, and make brief comments on the chromosomal evolution of the family based on the molecular phylogenies.

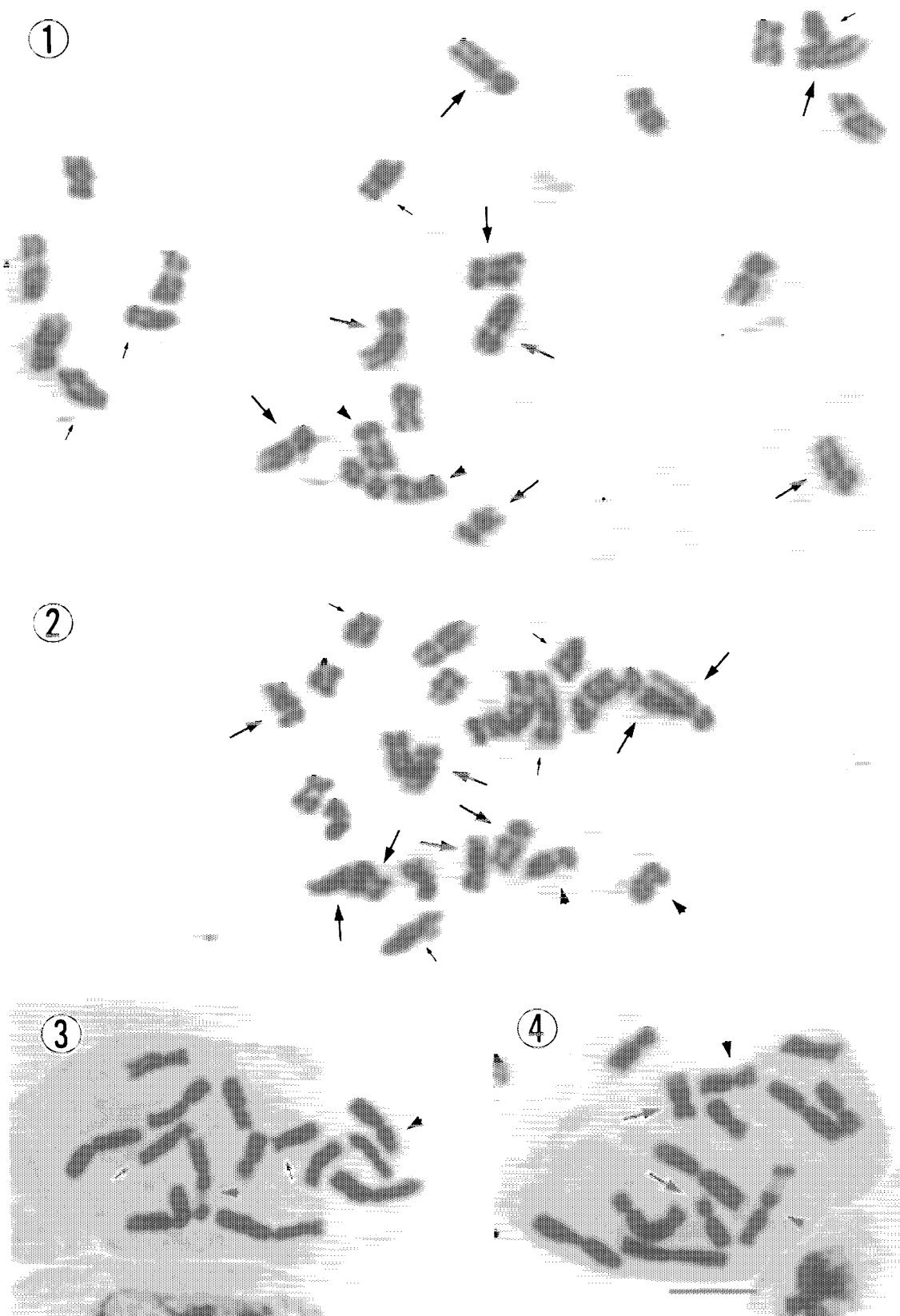
## Materials and Methods

The species investigated of *Croomia* and *Stemona* are shown in Table I along with their collection data. Somatic chromosomes of *Croomia* were examined using meristematic cells of root tips, and those of *Stemona* were examined using them of young leaves, respectively. Details of pretreatment, fixation, and staining for chromosome observations were described elsewhere (Oginuma & Nakata 1988; Oginuma *et al.* 1992). Categories of chromosome morphology on the basis of the position of centromeres followed Levan *et al.* (1964).

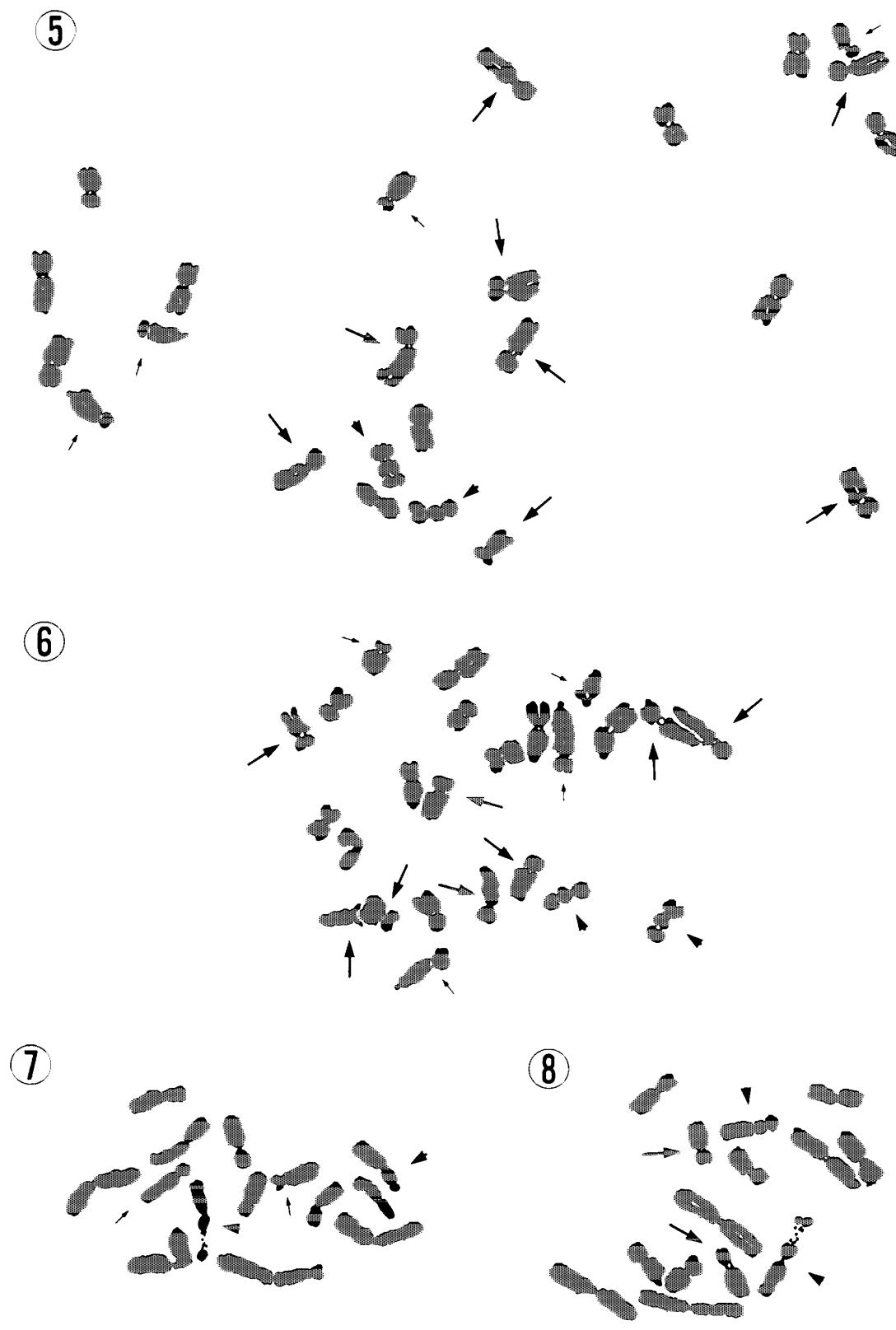
## Results and Discussion

Both *Croomia heterosepala* (Figs. 1 and 5) and

*C. japonica* (Figs. 2 and 6) have  $2n=24$  in 12 pairs. All the three species of *Croomia* have been reported to be  $2n=24$  (Table 2), suggesting the base number of this genus is considered to be  $x=12$ , although there is a report of  $2n=26$  for Chinese plants of *C. japonica* (Li 1986). The karyotypes at metaphase are nearly identical in the two examined species. In *C. heterosepala*, the chromosome length in the metaphase complement is gradual: the longest chromosome is about  $4.4 \mu\text{m}$  and the shortest one about  $2.3 \mu\text{m}$ . Ten of the 24 chromosomes have centromeres at median position (with an arm ratio 1.1-1.6), and ten at submedian (with an arm ratio 2.0-2.5), and four at subterminal position (with an arm ratio 3.1-4.5). In *C. japonica*, the chromosome length in the metaphase complement is gradual: the longest chromosome is about  $4.6 \mu\text{m}$  and the shortest chromosome about  $1.7 \mu\text{m}$ . Ten of the 24 chromosomes have centromeres at median position (with an arm ratio 1.0-1.6), ten at the submedian position (with an arm ratio 2.0-2.8), and four at subterminal position (with an arm ratio 3.1-4.5). The secondary constrictions are observed at the interstitial regions of the long



FIGS. 1-4. Somatic chromosomes of four species in Stemonaceae. 1: *Croomia heterosepala* ( $2n=24$ ). 2: *C. japonica* ( $2n=24$ ). 3: *Stemona japonica* ( $2n=14$ ). 4: *S. sessilifolia* ( $2n=14$ ). Large arrows indicate chromosomes with submedian centromeres. Small arrows indicate chromosomes with subterminal centromeres. Arrowheads indicate chromosomes with the secondary constrictions. Scale bar = 5  $\mu\text{m}$ .



Figs. 5-8. Drawings of somatic chromosomes of respective preceding photographs (Figs. 1-4). 5: *Croomia heterosepala* ( $2n=24$ ). 6: *C. japonica* ( $2n=24$ ). 7: *Stemona japonica* ( $2n=14$ ). 8: *S. sessilifolia* ( $2n=14$ ). Large arrows indicate chromosomes with submedian centromeres. Small arrows indicate chromosomes with subterminal centromeres. Arrowheads indicate chromosomes with the secondary constrictions. Scale bar = 5  $\mu\text{m}$ .

TABLE 2. Chromosome numbers of Stemonaceae *s.l.*

Species	<i>x</i>	References*
<i>Croomia</i>	12	
<i>C. heterosepala</i> (Backer) Okuyama		1
<i>C. japonica</i> Miq.		1
<i>C. pauciflora</i> (Nutt.) Torr.		2
<i>C. pauciflora</i> (Nutt.) Torr.		3
<i>Stemona</i>	7	
<i>S. japonica</i> Miq.		1, 4
<i>S. sessilifolia</i> Miq.		1
<i>S. tuberosa</i> Lour.		4
<i>Stichoneuron</i>	9	
<i>S. caudatum</i> Ridley		3
<i>Pentastemona</i>	7	
<i>P. erégia</i> (Schott) Steenis		3

\* 1; present study, 2; Li (1986), 3; Duryfjes (1991), 4; Suzuka and Koriba (1949).

arms in a pair of small-sized chromosomes in both species (indicated with arrowheads in Figs. 5 and 6).

In *Stemona japonica*, *S. sessilifolia* and *S. tuberosa*, chromosome numbers are  $2n=14$  and agree to the previous report on *S. japonica* (Suzuka & Koriba 1949), suggesting that the base number of the genus is  $x=7$ . The karyotypes at metaphase of *S. japonica* (Figs. 3 and 7) and *S. sessilifolia* (Figs. 4 and 8) are slightly different. In *Stemona japonica*, chromosome length in the metaphase complement is gradual: the longest chromosome is about 6.3  $\mu\text{m}$  and the shortest one about 2.9  $\mu\text{m}$ . Twelve of the 14 chromosomes have centromeres at median position (with an arm ratio 1.1-1.6) and two at subterminal (with an arm ratio 4.0-4.5). In *S. sessilifolia*, chromosome length in the metaphase complement is gradual: the longest chromosome is about 6.3  $\mu\text{m}$  and shortest one is about 2.9  $\mu\text{m}$ . Ten of the 14 chromosomes have centromeres at median position (with an arm ratio 1.1-1.6), two chromosomes at median-submedian position (with an arm ratio about 1.7), and two chromosomes at submedian position (with an arm ratio 2.0-2.5). The

secondary constrictions are observed at the interstitial regions of the short arms in a pair of medium-sized chromosomes in both species (indicated with arrowheads in Figs. 7 and 8).

DNA-based phylogenies cited above more or less differ in the adopted genes, taxa, and phylogeny producing procedures, but unequivocally support the monophyly of Stemonaceae *s.str.*, and place it in the Pandanales (in the sense of APG, 1998). A phylogeny based on *rbcL*, *atpB* and 18S rDNA (Soltis *et al.* 2000) places Stemonaceae *s.str.* the sister to Velloziaceae, but does not include *Pentastemona*. The phylogeny based on *rbcL* and *atpB* (Caddik *et al.* 2000) supports the position of *Pentastemona* within Stemonaceae as the basalmost clade, and places Stemonaceae sister to well-supported Pandanaceae/Cyclanthaceae clade. Although the position of *Croomia* in Stemonaceae is not identified in the molecular trees, it is not likely to be the basalmost clade of the family, because the derivation of the pentamerous flowers of *Pentastemona* from dimerous ones would contradict with several lines of morphological evidence. An examination of floral structure (T. Fukuhara in

prep.) indicates the flowers of *Pentastemonia* could have been derived through a simple deletion of a pair of a tepal and a stamen from trimerous flowers, which are common in monocots. The dimerous flower is better interpreted as a synapomorphy of Stemonaceae s.str., rather than the primitive state of the family. *Croomeia* shares with *Stichoneuron* many floral characters (van Heel 1992), including apical placenta, a probable synapomorphy.

Therefore, with available information, the parsimonious hypothesis is that  $x=9$  and  $x=12$  have derived from  $x=7$ , which is shared by *Pentastemonia*, the probable basalmost ingroup, and *Stemonia*. The base number of  $x=7$  is also found in Velloziaceae, which some molecular phylogenies regard as the closest relative of Stemonaceae. However, this similarity probably represents a parallism. *Acanthochlamys* P. C. Kao, the basalmost clade inferred from *rbcL* gene (Behnke *et al.* 2000) has  $2n=38$  (Kao *et al.* 1993), and  $x=8$  has been suggested as the primitive state of the family excluding *Acanthochlamys* (Melo *et al.* 1997). Further karyomorphological information from *Stichoneuron* and *Pentastemonia* would confirm and improve the current conclusion.

Kyoto Herbal Garden of Takeda Chemical Industries, Ltd. generously allowed us to collect meristems of *Stemonia* species. We cordially thank Prof. H. Tobe (Kyoto University) for his support throughout the study, Dr. M. N. Tamura (Osaka City University) for useful comments, and Mr. I. Watanabe (Takeda Chemical Industries, Ltd.) for his help at sampling.

## References

APG (The Angiosperm Phylogeny Group) 1998. An ordinal classification for the families of flowering plants. Ann. Missouri Bot. Gard. 85: 531-553.

Behnke, H. D., J. Treutlein, M. Wink, K. Kramer, C. Schneider & P. C. Kao. 2000. Systematics and evolution of Velloziaceae, with special reference to sieve-element plastids and *rbcL* sequence data. Bot. J. Linn. Soc. 134: 93-129.

Caddick, L. R., P. J. Rudall, P. Wilkin & M. W. Chase. 2000. Yams and their allies: systematics of Dioscoreales. In Wilson, K. L. & D. A. Morrison D. A. (eds.), Monocots: systematics and evolution, pp. 475-487. CSIRO, Melbourne.

Chase, M. W., D. E. Soltis, P. S. Soltis, P. J. Rudall, M. F. Fay, W. J. Hahn, S. Sullivan, J. Joseph, M. Molvray, P. J. Kores, T. J. Givnish, K. J. Sytsma & J. C. Pires. 2000. Higher-level systematics of the monocotyledons: an assessment of current knowledge and a new classification. In Wilson, K. L. & D. A. Morrison. (eds.) Monocots: systematics and evolution, pp. 3-16. CSIRO, Melbourne.

Duyfjes, B. E. E. 1991. Stemonaceae and Pentastemonaceae; with miscellaneous notes on members of both families. Blumea 36: 239-252.

\_\_\_\_\_. 1992. Formal description of the family Pentastemonaceae with some additional notes on Pentastemonaceae and Stemonaceae. Blumea 36: 551-552.

Fuse, S. & M. N. Tamura. 2000. A phylogenetic analysis of the plastid *matK* gene with emphasis on Melanthiaceae sensu lato. Plant Biol. 2: 415-427.

Heel, W. A. van 1992. Floral morphology of Stemonaceae and Pentastemonaceae. Blumea 36: 481-499.

Kao, P. C., Y. Tang & W. H. Guo. 1993. A cytological study on *Acanthochlamys bracteata* P. C. Kao (Acanthochlamyaceae). Acta Phytotax. Sinica 31: 42-44.

Kubitzki, K. 1998a. Pentastemonaceae. In Kubitzki, K., H. Huber, P. J. Rudall, P. S. Stevens & T. Stutzel. (eds.), The families and genera of vascular plants III: 404-406.

\_\_\_\_\_. 1998b. Stemonaceae. In Kubitzki, K., H. Huber, P. J. Rudall, P. S. Stevens & T. Stutzel (eds.), The families and genera of vascular plants III: 422-425. Springer, Berlin.

Levan, A., K. Fredga & A. A. Sandberg. 1964. Nomenclature for centromeric position of chromosomes. Hereditas 52: 201-220.

Li, L-C. 1986. Chromosome observations of some plants of China. Guihaia 6: 99-105.

Melo, N. F. de, M. Guerra, A. M. Benko-Iseppon & N. L. de Menezes. 1997. Cytogenetics and cytotaxonomy of Velloziaceae. Pl. Syst. Evol. 204: 257-273.

Oginuma, K. & M. Nakata. 1988. Cytological studies on phanerogams in southern Peru, I. Karyotype of *Acaena ovalifolia*. Bull. Natl. Sci. Mus., Ser. B 14: 53-56.

July 2001

OGINUMA *et al.*: Karyomorphology of Stemonaceae

63

....., G. Ibarra-Manríquez & H. Tobe. 1992. Chromosomes of *Tuxtla pittieri* (Asteridaceae; Heliantheae). *Acta Phytotax. Geobot.* 43: 135-137.

Qiu, Y. L., J. Lee, F. Bernasconi-Quadroni, D. E. Soltis, P. S. Soltis, M. Zanis, E. A. Zimmer, Z. Chen, V. Savolainen & M. W. Chase. 1999. The earliest angiosperms: evidence from mitochondrial, plastid and nuclear genomes. *Nature* 402: 404-407.

Soltis, D. E., P. S. Soltis, M. W. Chase, M. E. Mort, D. C. Albach, M. Zanis, V. Savolainen, W. Hahn, S. B. Hoot, M. F. Fay, M. Axtell, S. M. Swensen, L. M. Prince, W. J. Kress, K. C. Nixon & J. S. Farris. 2000. Angiosperm phylogeny inferred from 18S rDNA, *rbcL*, and *atpB* sequences. *Bot. J. Linn. Soc.* 133: 381-461.

Suzuka, O. & S. Koriba. 1949. Chromosome numbers of medical plants I. *Japanese Jour. Pharmacog.* 3: 68-74. (In Japanese).

Received December 18, 2000; accepted February 7, 2001